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(54) Quantitative Analysis Apparatus and Method

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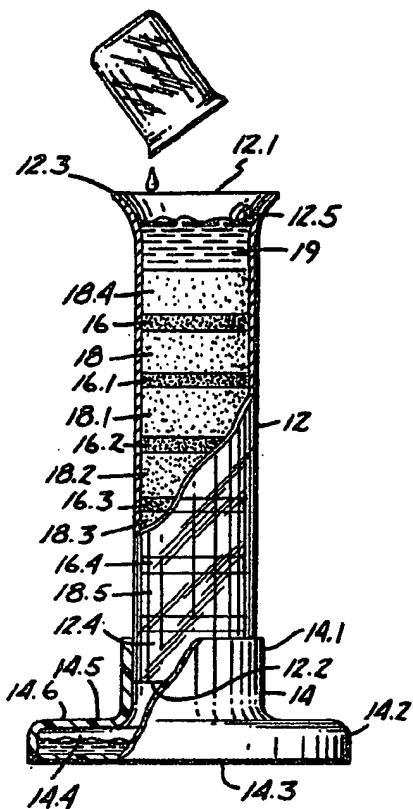


Fig. 1

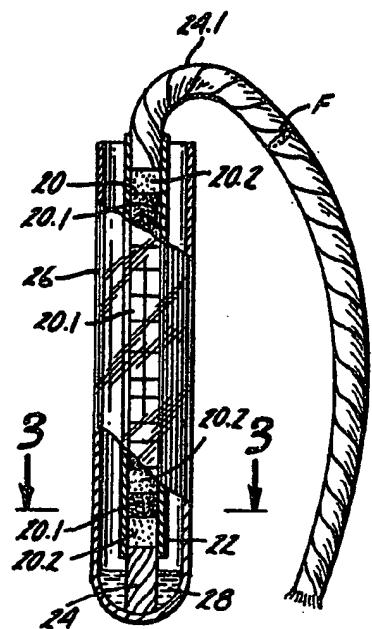


Fig. 2

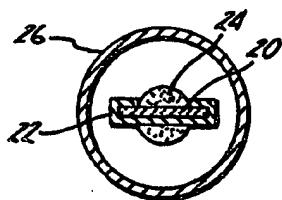


Fig. 3

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Fig.5

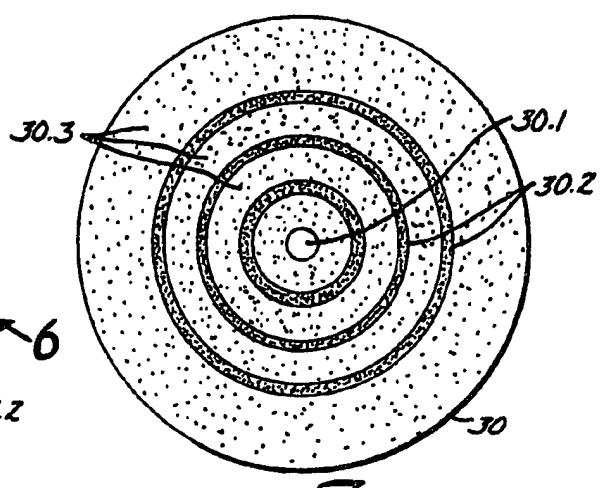
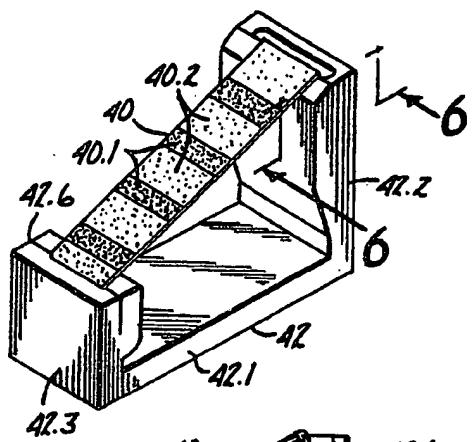


Fig.4

Fig.6

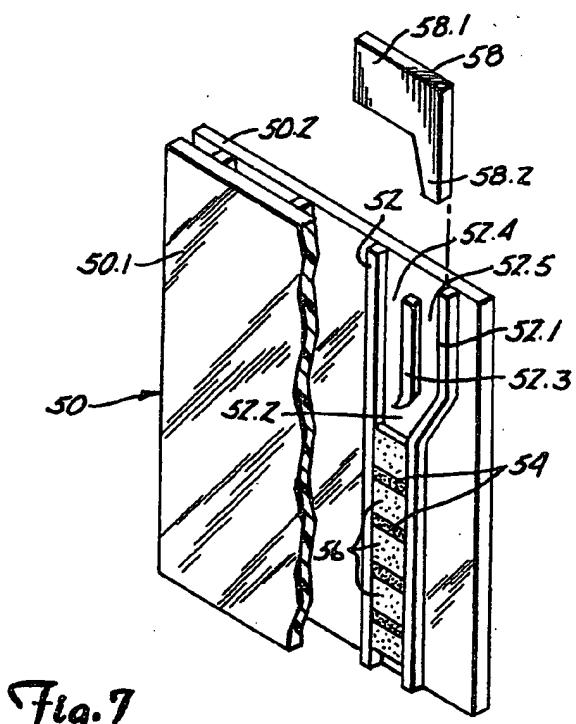
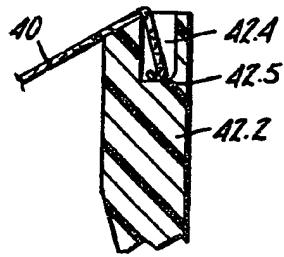


Fig.7

Intel & System

ABSTRACT

A method and apparatus for the quantitative determination of an analyte in a liquid employs a liquid-permeable solid medium defining a liquid flow path. The medium includes a number of reactant-containing reaction zones spaced apart along the flow path and in which reaction occurs with the analyte or an analyte derivative (e.g., a labeled analyte) to result in the formation of a predetermined product. Detector means are employed to detect analyte, analyte derivative, reactant or predetermined product in the reaction zones, the number of such zones in which such detection occurs indicating the amount of analyte in the liquid.

QUANTITATIVE ANALYSIS APPARATUS AND METHOD

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FIELD OF THE INVENTION

The invention is in the field of quantitative chemical analysis, and is particularly useful in the detection and analysis of small amounts of chemical substance in such biological fluids as milk, blood, urine, etc.

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DESCRIPTION OF THE PRIOR ART

Procedures for quantitatively determining the concentration of chemical substances in solutions are legion. Many of these procedures are long and tedious, and are highly susceptible to human error. Many procedures involve the reaction of the chemical moiety - the analyte - to be detected with a reactant to form a product, the procedures including a step of determining the amount of reactant that is consumed (e.g., as in titrations), or the amount of product that is produced (e.g., as by measuring the absorption of light by the product of a chromogenic reaction), or as by measuring the amount of the chemical moiety or reaction product that can be separated from the solution (e.g., by distillation), etc. Some quantitative analysis procedures, such as are used in radioimmunoassays, involve competitive reactions between labeled analytes (e.g., labeled with radioisotopes of iodine, enzymes, or fluorescent, chromogenic or fluorogenic molecules) in known quantities and unknown amounts of

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1 unlabeled analytes, the amount of analyte in the un-  
known solution being related to the measured radioac-  
tivity or other property of a specimen resulting from  
the test after suitably separating the reacted or  
bound analyte from the unreacted or unbound analyte,  
or through properties of the bound and unbound labeled  
analyte that permit them to be distinguished. Many of  
such procedures involve changes in color (as when  
chemical indicators are employed that respond by color  
10 changes to differences in hydrogen ion concentration),  
or in turbidity (as when the procedure involves the  
formation of a solid reaction product).

20 Certain analyses involve the passage of a  
fluid, such as air, through a column containing a re-  
actant which may change color upon contact with an  
ingredient of the air. For example, U.S. Patent  
3,286,506 describes a gas analyzing technique in which  
a measured amount of gas is passed through a glass  
cartridge containing an indicator, the amount of gas  
to be detected being proportional to the amount of  
indicator within the column that changes color. Sim-  
ilar devices are shown in U.S. Patents 3,312,527 and  
3,545,930.

30 There is a recurring trend in the field to pro-  
vide analytical procedures which are characterized by  
speed, simplicity, and by a reduction in the vulnar-  
ability of such procedures to human error. Simple,  
rapid tests, for example, have been marketed for de-  
termining the approximate level of blood sugar for  
diabetics. Such tests, however, often are relatively  
imprecise. It would be highly desirable to provide a  
quantitative test for chemical moieties that on the  
one hand would be characterized by high sensitivity  
and that yet on the other hand would be characterized  
by simplicity, rapidity and relative freedom from  
human error.

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SUMMARY OF THE INVENTION

In one embodiment, the invention provides an apparatus for the quantitative analysis of a chemically reactive substance (hereafter referred to as an "analyte"), in a carrier fluid such as a liquid. The apparatus includes a fluid-permeable solid medium that has a predetermined number of successive, spaced reaction zones and which defines a path for fluid flow sequentially through such zones. "Fluid" herein is typified as a liquid. Predetermined quantities of a reactant are bound to the solid medium in such zones and are capable of reaction with the analyte or with an analyte derivative, to result in the formation of a predetermined product. The apparatus may further include detector means for detecting, in the spaced zones, the presence of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant. In addition, the apparatus may include means for suppressing the detectability of trace amounts of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant.

As used herein, the terms "reactant", "reactive" and the like when used in connection with the reaction between the analyte or its derivative and the reactant refers to the ability of the reactant to react, by covalent or hydrogen bonding or by any other means, with the analyte or its derivative to form or result in the formation of a predetermined product. That is, such terms are used in their broadest sense as referring to the ability of the reactant to in any way act upon, be acted upon, or interact with the analyte or analyte derivative in a manner that detectably alters the analyte or its derivative, the reactant or

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1 both to thereby result in the formation of a reaction  
product. Similarly, "reaction product" means any  
product resulting from the reaction of the analyte or  
its derivative and the reactant and that is detectably  
different from both. "Analyte derivative" means a  
chemical moiety derived from an analyte, and desirably  
is a tagged or labeled form of the analyte as may be  
employed in analytical procedures involving competing  
reactions between an analyte and its tagged or labeled  
10 derivative.

In the apparatus of the invention, the reactant  
is bound to the permeable solid medium in the succes-  
sive, spaced zones through which the analyte passes.  
A procedure employing the apparatus may take the form  
in which the analyte or its derivative, as it passes  
through the reaction zones, becomes bound to the re-  
actant and the presence of the analyte or its deriva-  
tive within the reaction zones is detected, as by  
color change or the like. Similarly, in a slightly  
20 modified embodiment, the analyte or its derivative may  
react with the reactant to result in the formation of  
a product that itself remains bound in the reaction  
zones, and the product itself is then detected. In  
these embodiments, one may determine with considerable  
precision the concentration of the analyte by detect-  
ing how many of the successive reaction zones, begin-  
ning with the upstream zone, show the presence of the  
analyte or its derivative, or of the product resulting  
30 from the reaction between the reactant and the analyte  
or analyte derivative. In another embodiment, the  
reactant that is bound to the permeable solid medium  
may itself be capable of detection by suitable detec-  
tion means and may be disabled from such detection  
when reacted with an analyte or analyte derivative.  
In this manner, as the analyte or analyte-analyte

1 derivative composition passes through successive reaction zones, the reactant in the successive zones is disabled from such detection until substantially all of the analyte or analyte-analyte derivative composition has been exhausted, while remaining downstream reaction zones still contain reactant that can be detected. In a modified form, the reaction between the analyte or analyte derivative and the reactant may cause the latter to become unbound from the solid  
10 medium to which it was attached and hence be washed from the successive zones. When the analyte or analyte derivative or both has thus been exhausted, subsequent or downstream reaction will display reactant that is yet bound to the permeable medium and which can be detected. In such embodiments, one may count the number of zones in which the reactant has been disabled beginning with the upstream zone.

As used herein, "analyte" refers not only to the particular chemical moiety for which analysis is desired, but also to chemical moieties that are reaction products of the moiety to be determined with another chemical moiety. For example, a biological fluid containing an unknown amount of a chemical moiety may be reacted in solution or otherwise with another chemical moiety to provide a product, the concentration of which is related to the initial concentration of the chemical moiety to be measured. The resulting product, then, may become the "analyte" for use in the apparatus and method of the invention.  
20 Accordingly, "analyte" refers to any chemical moiety which is to be measured quantitatively.

In a preferred embodiment, the invention employs immunochemical reactions in which the analyte and the reactant represent different parts of a specific ligand-antibody (antiligand) binding pair.

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DESCRIPTION OF THE DRAWINGS

Figure 1 is a broken-away view, in partial cross-section, showing an apparatus of the invention;

Figure 2 is a broken-away view, in partial cross-section, showing another apparatus of the invention;

Figure 3 is a cross-sectional view taken along line 3-3 of Figure 2;

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Figure 4 is a plan view of another embodiment of an apparatus of the invention;

Figure 5 is a perspective view of yet another test apparatus of the invention;

Figure 6 is a broken-away cross sectional view taken along line 6-6 of Figure 5; and

Figure 7 is a perspective, broken-away view of another test apparatus of the invention.

DETAILED DESCRIPTION

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With reference to Figure 1, a transparent, hollow column of glass or the like is designated 12 and has open top and bottom ends (12.1, 12.2). The top end (12.1) preferably is flared outwardly as shown at (12.3). A standard (14) is provided at the bottom end of the column, and may have a hollow, upstanding portion (14.1) into which the bottom portion (12.4) of the column may be snugly fitted as by a press fit.

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**B** The standard includes a relatively wide bottom portion (14.2) having a flat, horizontal surface <sup>(14.3)</sup> such as a table. The interior (14.4) of the standard preferably is hollow, and an upper wall (14.5) of the standard preferably is provided with a breathing aperture (14.6) so as to permit air to escape from the column when liquid is poured into the upper end (12.1) of the column. The aperture (14.6) may, if desired, be fitted with a loose, porous plug, such as a cotton plug, to retard leakage from the device when it has been disposed of in a trash container or the like. It

1 may also, if desired, be fitted with a flexible tubing  
which may connect it with a pump (e.g., peristaltic,  
syringe drive withdrawal, etc.) typifying flow control  
means to control the flow rate of the liquid through  
the assay column.

**B** Within the column are positioned successive,  
spaced reaction zones (16, 16.1, 16.2, 16.3, <sup>16.4</sup> etc.),  
occupied by a permeable solid medium such as beaded  
agarose, beaded polyacrylamide, porous glass, cellu-  
lose or other materials permeable to liquid and com-  
patible with the analyte, analyte derivative, reactant  
and detector means. To the medium in the reaction  
zones is bound a reactant, as will be described more  
fully below. The interior of the column, as will now  
be understood, describes a generally vertical liquid  
flow path, and the permeable solid medium positioned  
in the reaction zones desirably occupies the entire  
cross-section of the flow path. Between the spaced  
reaction zones are positioned preferably nonreactive  
20 spacer layers (18, 18.1, 18.2, 18.3 and so on) of a  
liquid-permeable solid medium through which liquid may  
flow, the spacer layers preferably being in intimate  
contact with the reaction zones. The spacer layers  
desirably are of the same permeable solid medium as  
the reaction zones, and, preferably, spacer layers  
18.4, 18.5 are provided at the top and bottom of the  
columns as well so that each reaction zone is sand-  
wiched between spacer layers. At its upper end, the  
column (12) may be provided with an aperture (12.5)  
30 spaced a given distance above the spacer layer (18.4)  
so as to provide a predetermined volume between the  
aperture and top surface of the spacer layer. In a  
known manner, as a liquid (represented as (19) in the  
drawing) is poured into the open upper end (12.1) of  
the column, it will occupy the open volume at the top

1      **B** of the column and any amount of the liquid ~~(20)~~ in excess of that desired may escape outwardly through the aperture (12.5), thereby insuring that no more than a given, predetermined amount of the liquid passes downwardly in the column. The space <sup>19</sup> ~~(20)~~ may, if desired, be filled or partially filled with a porous, nonreactive material such as glass wool or similar material to avoid splashing of the liquid within the upper end of the column.

10      To the liquid-permeable solid medium within the spaced reaction zones (16, 16.1, etc.) is bound a reactant that is reactive with a given analyte or analyte derivative to form a product, all in accordance with the above definitions and as exemplified herein. As a typical example, the reactant and analyte may be so chosen that the analyte or its derivative becomes chemically attached to the reactant as the solution ~~(20)~~ of analyte or analyte and analyte derivative solution (the "test solution") passes downwardly through the column, care being taken that the total amount of reactant within the column is in excess of that required to so react with the expected quantity of analyte and analyte derivative in the solution. After the test solution has begun its passage downwardly through the column, a wash solution, typically distilled and deionized water, optionally may be poured into the open end of the column to further aid the downward passage of the test solution through the column. Finally, an indicator or detector material that detects the presence of analyte or analyte derivative, reaction product or reactant, as by causing a color change within the zones (16, 16.1 and so on) may be poured into the upper end of the column. As the test solution flows downwardly through the column, predetermined amounts of the analyte or its derivative are reacted with or bound to the reactant in each such

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1       layer until the analyte or its derivative have been  
exhausted from the solution. The concentration of  
analyte in the solution can be determined by merely  
counting the number of successive zones, beginning at  
B the top of the column, that have changed color. In  
another embodiment, the reactant that is bound to the  
medium in the zones (16, 16.1 and so on) may be de-  
activated or disabled by reaction with the analyte  
or analyte derivative or both, and the detector which  
10      is employed may determine, as by a color change, the  
presence of nondisabled reactant. In this embodiment,  
the reactant in successive zones contacted by the test  
solution will be disabled until analyte and analyte  
derivative have been exhausted from the solution.  
Upon detecting which of the reaction zones contain  
reactant that has not been disabled, one may determine  
the concentration of analyte in the solution by count-  
ing the number of zones beginning at the top of the  
column in which reactant is not detected. Of course,  
20      in this embodiment as in the embodiment set out above,  
one may also count the number of zones beginning with  
the bottom of the column as well.

Another physical embodiment of the apparatus  
of the invention is shown in Figures 2 and 3 in which  
"wicking", or upward capillary flow of a liquid  
through a strip of filter paper or similar material is  
employed, the strip having successive, spaced reaction  
zones. In this embodiment, the permeable solid medium  
may take the form of a strip of filter paper, which is  
30      designated generally as (20) in Figures 2 and 3.  
Within spaced zones (20.1) of the filter paper strip  
is bound a reactant, as above-described, the spaced  
zones being separated by spacer layers or sections  
(20.2). One method of preparing the strip (20) in-  
volves binding a reactant to small, individual rec-  
tangular paper filter pieces, and then alternating

1 these pieces, which form the reaction zones, with  
similar pieces of filter paper that do not contain the  
reactant, the alternating pieces of filter paper being  
held together, for example, by a thin strip of ad-  
hesive tape. Other, more sophisticated methods of  
manufacture will be apparent to those skilled in the  
art.

10 As shown in Figures 2 and 3, the strip (20) of  
filter paper may be positioned in an elongated plastic  
holder (22) having a generally C-shaped cross-section.  
The bottom of the holder is adapted to receive the end  
of a wick (24) which may consist of twisted strands of  
cotton or other fibrous material. A similar wick  
(24.1) is received in the upper end of the plastic  
holder. The ends of the wicks (24, 24.1) contact the  
ends of the filter strip (20). As shown in Figure 2,  
the upper and lower sections of the filter paper strip  
which come into contact with the wicks (24, 24.1), are  
spacer layers (20.2) so that each reaction zone (20.1)  
is sandwiched between spacer layers (20.2). The fil-  
ter paper strip and holder are adapted for insertion  
into a test tube (26) or other container so that the  
lower wick (24) contacts the bottom of the test tube  
and the upper wick (24.1) extends out of the test tube  
and then downwardly toward its bottom, all as shown in  
Figure 2. A test solution (28) placed in the bottom  
of the test tube (26) is thus caused to flow by capil-  
lary action upwardly through the length of the filter  
paper strip, successively contacting the reaction  
30 zones (20.1) in a manner analogous to the flow of test  
solution through the column depicted in Figure 1. As  
will be described more fully below, the filter paper  
strip and holder can be moved from one test tube to  
another so that different solutions can be caused to  
sequentially flow through its length.

1 Referring now to Figure 4, an apparatus of the  
invention is embodied in a disc of permeable, solid  
medium such as filter paper, porous glass, etc. (30).  
The disc (30) may be placed horizontally in a suitable  
container such as a petri dish. At its center, the  
disc (30) is provided with a well (30.1) to receive a  
test solution or other solution. Reaction zones,  
spaced radially from the well (30.1), are shown as an-  
nular rings (30.2), and are separated from one another  
10 by spacer layers also in the form of annular rings  
(30.3). Spacer layers preferably form the innermost  
and outermost rings of the disc. The reaction zones  
(30.2) and the spacer layers (30.3) are concentric.  
Test solution that is admitted to the central well  
(30.1) is thus carried radially outwardly of the well  
by capillary action or by diffusion, aided by centrif-  
ugal force if desired, the test solution successively  
passing through the spaced reaction zones (30.2).

20 Figures 5 and 6 show another embodiment of a  
device of the invention. The device includes a filter  
paper strip (40) similar to that of Figures 2 and 3  
and containing spaced reaction zones (40.1) separated  
by spacer layers (40.2). A holder, preferably of  
plastic, is designated (42) and has a flat base (42.1)  
with upwardly extending legs (42.2, 42.3) carried at  
its ends. The leg (42.2) is provided with an upwardly  
open well (42.4) into which may be inserted the upper  
end of the filter paper strip (40), care being taken  
that the end (42.5) of the filter paper extends down-  
30 wardly to the floor of the well. The strip of filter  
paper extends obliquely downwardly from the well, and  
its lower end is captured in a slot (42.6) formed in  
the leg (42.3). In use, the test solution or other  
solution is placed in the well (42.4), and is carried  
downwardly of the strip by both gravity and capillary

1 action, the solution sequentially encountering the  
spaced reaction zones (40.1).

Figure 7 shows yet another embodiment of a device of the invention which can be used for multiple concurrent tests. The device, designated (50), includes a pair of spaced plates (50.1, 50.2). Referring to the right-hand portion of Figure 7, the space between the plates is divided into generally vertical channels by means of elongated spacers (52, 52.1). As shown in the drawing, the channel (52.2) formed by the spacers has a wide upper section and a narrow lower section. The lower section is provided with a series of vertically spaced reaction zones (54) comprising a liquid-permeable solid medium to which is bound a reactant, the medium being any of those described above. Between the reaction zones, are placed spacer layers (56), the spacer layers sandwiching between them the reaction zones (54). Between the spacers (52, 52.1), at the upper end of the channel is placed an elongated vertical divider (52.3) which divides the upper portion of the channel into two sections (52.4) and (52.5). A plug (58), which may be made of the same material as the spacers, has an upper, finger-gripping portion (58.1) and a lower, tapering plug portion (58.2) adapted to be inserted in the channel (52.5). The flat surfaces of each of the spacers and plug, of course, contact the facing surfaces of both glass plates to prevent leakage of material from the channels.

In use, a solution such as a test solution is poured into the upper end of the channels formed by the spacers (52, 52.1), and the plug (58) is then inserted to provide an air-tight upper seal in the one channel (52.5). As a result, liquid in the other channel (52.4) preferentially flows downwardly through the reaction zones and spacer layers. When the liquid

1 level in the channel (52.4) falls below the lower end  
of the spacer (52.3), air can bubble upwardly through  
the channel (52.5), permitting the contents of that  
channel to empty downwardly through the reaction zones  
as well. In this manner, the sequential flow of liq-  
uid, first from channel (52.4) and then from channel  
(52.5), is rendered automatic. Preferably, one of the  
plates (e.g., plate 50.1) is transparent so that the  
results of any color change in the reaction zones may  
readily be observed. The other plate (50.2) may be  
transparent or may be of an opaque white or other  
light color to serve as a background against which  
color changes can readily be seen.

#### ANALYTES-REACTANTS

20 Analytes that can be detected in accordance  
with the present invention include substantially all  
chemical substances that are reactive with a reactant  
to form a product, as above discussed. It will be  
understood that the invention is not limited to any  
particular analyte or reactant, but is useful for sub-  
stantially any analyte-reactant combination.

Many analytes may be analyzed simply by adapt-  
ing known chemical reactions to the invention.

30 For example, carbon dioxide may be analyzed  
with phenolphthalein at a slightly alkaline pH. Cal-  
cium ion may be analyzed utilizing a reactant compris-  
ing calmodulin and mammalian phosphodiesterase or  
another calmodulin-sensitive enzyme (Maekawa and Abe,  
Biochemical and Biophysical Research Communications  
97:621 (1980)). Ferrous ion may be analyzed utiliz-  
ing, as a reactant, a ferrocene derivative (Katz, et  
al, J. Am. Chem. Soc. 104:346 (1982)). A large number  
of additional examples may be selected from the list  
of organic analytical reagents compiled by John H. Yoe  
in Handbook of Chemistry and Physics, p.D 126-129,

1 57th Edition, Robert C. Weast, Ed., CRC Press, Cleve-  
land, 1976, and in other references cited therein.

Typical analyte-reactant pairs selected from  
the field of organic chemistry similarly may be chosen  
by adapting known chemical reactions to the inven-  
tion. For example, almost any phenol may be analyzed  
with Gibbs Reagent (2,6-dichloro-p-benzoquinone-4-  
chlorimine) (Dacre, J., Analytical Chemistry 43:589  
(1971)). A reagent for Indoles is p-dimethylamino-  
10 benzaldehyde (Fieser and Fieser, Reagents For Organic  
Synthesis, Volume 1, p.273, John Wiley & Sons, Inc.,  
New York, (1967)). The last-mentioned reference also  
shows the use of phenylhydrazine as a reactant for  
cortisone and similar steroids, and the use of sulfo-  
acetic acid as a reactant in the Liebermann - Burchard  
test for unsaturated sterols. Amino acids and ammoni-  
um salts may be analyzed using the reagent ninhydrin.  
(indane-1,2,3-trione hydrate) (Pasto, et al Organic  
Structure Determination, p.429, Prentice-Hall, Inc.,  
Englewood Cliffs, New Jersey, 1969). Reducing sugar  
20 may be measured with Red Tetrazolium (2,3,5-triphenyl  
-2H-tetrazolium Chloride) (Fieser, Organic Experi-  
ments, p.135, Raytheon Education Co., Lexington,  
Massachusetts, 1968).

Various other analyte reagent pairs may be se-  
lected from the field of chemistry for adaptation to  
the invention from such reference works as: Schuurs,  
et al, U.S. Patent 3,654,090 (Enzyme-Linked Immuno-  
sorbent Assay); Kay, U.S. Patent 3,789,116 (Fluores-  
30 cent Labeled Antibody Reagents); Rubenstein, et al,  
U.S. Patent 3,817,837 (Homogeneous Enzyme Immuno-  
assay); Ling, U.S. Patent 3,867,517 (Radioimmuno-  
assay); Giaever, U.S. Patent 3,906,490 (Radial Immuno-  
diffusion); Ullman, U.S. Patent 3,996,345 (Floures-  
cence Quenching Homogenous Immunoassay); Maggio, U.S.  
Patent 4,233,402 (Enzyme Channeling Homogeneous Enzyme

Immunoassay); Boguslaski, et al, Canadian Patent 1,082,577 (Hapten-Cofactor Homogeneous Enzyme Immunoassay); Schonfeld, H., Ed., "New Developments in Immunoassays", Antibiotics and Chemotherapy, Volume 26, 1979; O'Sullivan, et al, "Enzyme Immunoassays: A Review", Annals of Clinical Biochemistry 16:221 (1979); Schuurs, et al, Enzyme Immunoassay, Clin. Chim. Acta. 81:1 (1977); Feldmann, et al, Eds., First International Symposium On Immunoenzymatic Techniques, INSERM Symp.

10 No. 2, North Holland Publishing Co., Amsterdam, 1976; Williams, et al, Methods in Immunology and Immunochemistry, Volume 3, Academic Press, New York, 1971; and Yalow, et al, J. Clin. Invest. 39:1157 (1960).

20 Yet other analyte-reactant pairs may be found in: reference works such as Feigl, F., Spot Tests in Inorganic Analysis, 6th edition, Elsevier Publishing Co., New York, 1972; Feigl, Fritz, Spot Tests in Organic Analysis, 7th edition, Elsevier Publishing Co., New York, 1966; Snell, F. and Snell, C., Colorimetric Methods of Analysis, Vols. 1-4AAA, Van Nostrand Reinhold Co., New York, 1967-74; and Braibanti, A., Ed. Bioenergetics and Thermodynamics: Model Systems - Synthetic and Natural Chelates and Macrocycles as Models for Biological and Pharmaceutical Studies, D. Reidel Publishing Co., Boston, 1980.

30 Of particular importance to the instant invention are those analyte-reactant combinations that form specific binding pairs of which one is an antibody and in which the other is a ligand to which the antibody is specific.

Such immunochemical reactant pairs are well-known in the art, and a wide variety of tests have been devised to detect the presence of quantity or

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1 both of an analyte, particularly when the analyte is  
present only in exceedingly small concentrations.  
Reference is made to the above-identified patents and  
publications.

DETECTORS

10 The detectors useful in the invention are capable of detecting the presence in the successive reaction zones, of analyte, analyte derivatives, reactants, or the predetermined reaction product, all as described above. The means of detection may take various forms. In the preferred embodiment, detection is signaled by a change of color, or a lack of a change of color, in the respective reaction zones of the apparatus. However, detection may be signaled by other means as well, such as by luminescence or fluorescence of the zones, radioactivity of the zones, etc. For many reactions, detection is signaled by a change in pH, and the detector may hence take the form of a pH color indicator such as phenolphthalein, Nile Blue A, Thymol Blue, and Methyl Violet. In other tests, one may detect the presence or absence of the appropriate chemical moiety in a reaction zone by observing whether a solid reaction product has settled upon the succeeding spacer layer. Various detector mechanisms are known to the art, and need not be described in detail. In the preferred embodiment, however, which makes use of immunochemical reaction between the analyte or the analyte and its derivative and the reactant, often very small concentrations of analyte are to be measured and accordingly a magnifying or amplifying mechanism may suitably be employed. One such mechanism makes use of enzymes to promote the reaction of a reaction product with a detector moiety to in turn provide a visual color indication. For example, the analyte to be tested may be provided in admixture with a known quantity of an analyte-glucose

1        oxidase conjugate as an analyte derivative, the  
spaced, sequential reaction zones of the apparatus  
containing an antibody specific to the analyte. A  
signal generating system, such as horseradish peroxy-  
dase bound to the antibody in the permeable solid  
medium in such zones and a chromogenic material such  
as o-dianisidine (added, with glucose, to the test  
solution) can be employed. The addition of the test  
solution, containing the analyte, the analyte-glucose  
10      oxidase conjugate, glucose, catalase and o-dianisidine,  
is then flowed through the apparatus, such as the  
column depicted in Figure 1. The analyte and the ana-  
lyte-glucose oxidase conjugate compete for binding  
sites on the bound antibody, thereby resulting in a  
color formation due to the reaction of the o-dianisi-  
dine with hydrogen peroxide produced by the glucose  
oxidase - catalyzed reaction of oxygen with glucose.  
Unreacted analyte and analyte-glucose oxidase conju-  
gate flow to successive zones until the admixture has  
20      been exhausted of analyte and analyte-glucose oxidase  
conjugate. A variety of modifications of this proce-  
dure, of course, are known to the art.

Example I

30      The chromogen 5,5' [3-(2-Pyridyl)-1,2,4-  
triazine-5,6-diyl]bis-2-furansulfonic acid, disodium  
salt, ("Ferene", a trademarked product of Chemical  
Dynamics Corp.) is used for the determination of serum  
iron in soluble assays through measurement of the  
absorbance at 593nm, at which wavelength any inter-  
ference from other pigments in the serum should be  
minimized. It may be covalently coupled to useful  
carrier derivatives through nitration, reduction,  
diazotization and diazonium coupling to proteins (such  
as albumin) immobilized on agarose beads, paper strips  
or other suitable permeable solid medium. This im-  
mobilized signal-generating reagent (chromogenic

1 chelating agent) is physically arranged in sequential spaced layers or bands (reaction zones) through which the test fluid will migrate.

20 Small columns are prepared from silanized Pasteur pipettes by breaking off both ends, attaching a short piece of tubing to the bottom (constricted) end and inserting glass wool plugs in the tube bottoms. The columns are packed by sequential insertion of layers of agarose-Ferene separated by layers of unmodified agarose. Typically, 0.4 ml of a 1:1 agarose suspension is applied directly above the support, followed by alternating layers of 50 microliters of a 1:1 suspension of agarose-Ferene and 0.2 ml of agarose suspension. After each addition to the column, the walls of the columns are rinsed with phosphate-buffered saline (PBS) and the solution above the gel is allowed to flow into the gel before addition of the next layer.

30 For use in the assay, the tubing at the bottom of the prepared column is connected to a peristaltic pump to control the flow rate of the assay. An appropriate dilution of test sample for iron analysis is applied to the column. The iron solution ("test" solution) is passed through the assay column at controlled flow rates, typically between 10 and 15 minutes for complete entrance. When all the solution is into the gel bed, the columns are rinsed with water. As the test solution flows through, color develops in some of the Ferene-containing reaction zones. The number of colored zones resulting is a function of the concentration of iron in the test solution.

Example II

A. The enzyme cholinesterase reacts with and is inhibited by toxic organophosphate and carbamate agents. Cholinesterase and the chromogenic sulphydryl

1 reagent 5,5'-Dithiobis-(2-nitrobenzoic acid) (Ellman's  
Reagent) are immobilized upon agarose beads, which are  
then assembled into columns according to Example I. A  
test solution (diluted blood serum) is added to the  
column and migrates through the reaction zones follow-  
ing which a solution <sup>of butyryl-thiobutyryl iodide</sup> ~~of butyryl-thiobutyryl iodide~~ is  
10 added. The zones retaining catalytically active  
cholinesterase will generate a yellow color through  
reaction of the thiobutyrine produced by the hydrolytic  
activity of the cholinesterase, with the immobilized  
Ellman's Reagent. The presence of reactive choline-  
sterase-inhibiting toxin in the test sample will re-  
sult in fewer colored bands, which will be in the  
downstream end region of the column.

20 B. Amino acids and other nucleophilic amine  
compounds are measured by use of the chromogenic re-  
agent 2,4-dinitrofluorobenzene (FDNB), which produces  
a yellow product upon reaction. A 0.1 ml aqueous  
sample, containing about 0.1-1.0 micromoles of amino  
analyte, is transferred to a siliconed glass vessel.  
The pH is adjusted to 7.0, if necessary, and two  
milligrams (25 micromoles) of NaHCO<sub>3</sub> is added and  
dissolved. Next is added 0.12 ml of 0.15% FDNB in  
absolute alcohol (1.5 micromoles). This solution is  
prepared fresh shortly before use. After the reaction  
has neared completion, it is analyzed for remaining  
FDNB (reactant) content by flow exposure to the assay  
system prepared according to Example I. In this case,  
a similar amine-containing analyte is immobilized in  
30 the zones in a permeable solid medium at a known con-  
tent (e.g., 0.1-0.25 micromoles per reaction zone).  
After rinsing with 50% ethanol in aqueous solution,  
the number of yellow reaction zones produced and re-  
maining after rinse will be inversely related to the  
amount of analyte in the test sample.

Example III

1 The IgG fraction from rabbit anti-penicilloyl-  
bovine gamma globulin was partially purified by pre-  
cipitation with 33% saturated ammonium sulfate. The  
precipitate was redissolved and dialyzed against phos-  
phate buffered saline (PBS). This IgG preparation was  
used for immobilizing antibodies onto beaded agarose.  
The agarose was suspended in dioxane, then reacted  
10 with carbonyldiimidazole. After being washed with  
dioxane, it was suspended in water, and then in  
aqueous borate buffer, pH 9.0. The IgG was then added  
to the activated agarose and the gel suspension  
stirred by rocking at 4° C for 2 days. After ex-  
tensive washing with PBS, the gel containing im-  
mobilized antibody was ready for use in the assay.

20 Small columns were prepared from silanized  
Pasteur pipettes by breaking off both ends, attaching  
short pieces of tubing to the bottom (constricted)  
ends and inserting glass wool plugs in the column bot-  
toms. The columns were packed by sequentially insert-  
ing alternating layers of agarose-IgG separated by  
layers of unmodified agarose. Typically 0.4 ml of 1:1  
agarose suspension was applied directly above the  
plugs, followed by alternating layers of 50 micro-  
liters of a 1:1 suspension of agarose-IgG (to form the  
reaction zones) and 0.2 ml of agarose suspension (to  
form spacer layers). After each addition, the walls  
of the columns were rinsed with PBS and the solution  
above the gel was allowed to flow into the gel before  
30 addition of the next layer.

For use in an assay, tubing at the bottom of  
the prepared column was connected to a peristaltic  
pump to control the flow rate of the assay. An  
appropriate dilution of penicilloyl-glucose oxidase  
("Pen-GO") (typically 0.1 microgram Pen-GO in 1 ml  
PBS) with or without known amounts of the analyte

1 (penicilloyl-epsilon amino caproate) (Pen-EAC), was  
applied to the column. The Pen-GO was prepared by  
reacting penicillin G with glucose oxidase in borate  
buffer, pH 9.0, for 2-3 days at 4° C. The Pen-GO  
solution was passed through the assay column at con-  
trolled flow rates, typically between 10 and 15 min-  
utes for complete entrance into the gel. When all the  
solution was into the gel bed, a detector solution was  
added to the column. The detector solution was pre-  
pared as follows: 0.20 ml of horseradish peroxidase  
(HRP) solution (2 mg/ml), 2 ml of 18% glucose solu-  
tion, 1 ml of 0.2 M phosphate buffer (pH 6.0) and  
0.100 ml of 1% o-dianisidine was diluted 1:10 in PBS  
and 1 ml or less was applied to the columns at the  
same flow rate as the previous solutions. Brown color  
developed in some of the reaction zones. Presence of  
the penicilloyl moiety in the Pen-GO solution results  
in the upper reactive zone or zones being lighter in  
color, with color being generated in zones further  
down the column.

10 20 This Example may be repeated for the analysis  
of serum albumin (a large protein molecule) by re-  
placement of the penicillin-glucose oxidase conjugate  
with an albumin-glucose oxidase conjugate.

Example IIIA

30 Peroxidase-labeled IgG prepared from rabbit  
antiserum against penicillin was immobilized in small  
strips of filter paper by the method reported in Ex-  
ample III. Catalase was bound to other, similar  
strips of filter paper. The first and second men-  
tioned strips were then cut into rectangular shapes to  
provide, respectively, reaction zones and spacer lay-  
ers. The small rectangular pieces of filter paper  
were then layed onto a strip of adhesive tape, alter-  
nating the reaction zones and spacer layers with edges  
of the sequential pieces of paper overlapping or at

1 least touching one another to provide a continuous capillary flow path.

Penicilloyl-glucose oxidase ("Pen-GO") in a solution of Human Serum Albumin ("HSA") was freeze-dried inside a test tube. Within another test tube, made of brown glass for protecting the contents from light, was freeze-dried a solution of o-dianisidine and glucose in phosphate-buffered saline at pH 6.0.

10 A short wick was attached to the bottom of the prepared filter paper strip described above, and a longer wick was placed in contact with the upper end of the strip. The strips themselves can be stored under refrigeration, and preferably are retained in a wet condition resulting from the preparation described above.

20 In one example of use, a test solution consisting of a measured volume of milk containing a known concentration of penicillin G is added to the test tube containing the freeze-dried Pen-GO, and the tube is shaken gently to mix the contents. The filter paper strip is then inserted into the tube with its upper, longer wick extending over the lip of the tube and then downwardly as shown in Figure 2. When the entire solution has been taken up by the strip (or, alternatively, when the solution reaches an arbitrary flow line marked on the upper wick and designated "F" in Figure 2) the filter paper strip is removed from the test tube and is placed in the brown glass tube to which previously has been added water to dissolve the 30 freeze-dried contents thereof. The latter solution similarly wicks upwardly through the filter paper strip, causing color development to occur in certain of the reaction zones as determined by the quantity of penicillin G in the initial test solution.

In this example, the penicillin G in the milk and the penicillin of the Pen-GO compete for binding

1 sites on the antibodies immobilized in the reaction  
2 zones of the filter paper strip. Of course, larger  
3 concentrations of penicillin G in the milk sample  
4 cause the penicillin G and the Pen-GO to migrate fur-  
5 ther through the filter paper strip. The presence of  
6 Pen-GO in any of the zones is indicated through the  
7 development of color from the reaction of  $H_2O_2$   
8 with o-dianisidine, the  $H_2O_2$  being formed from the  
9 glucose oxidase in the presence of glucose and oxygen,  
10 and as catalyzed by the peroxidase. The catalase in  
11 the spacer layers catalyzes the conversion of  $H_2O_2$   
12 into  $O_2$  and  $H_2O$ , and thus prevents migration of  
13  $H_2O_2$  from one reactive zone to another.

14 As with each of the apparatuses described here-  
15 in, the device of this example may be calibrated by  
16 determining how many of the reaction zones become  
17 colored as a result of the test procedure. For exam-  
18 ple, one of the reaction zones may change color only  
19 when the test solution (e.g., milk) contains at least  
20 9 nanograms of analyte (e.g., penicillin G) per ml.  
21 For a sample of milk containing an unknown concentra-  
22 tion of penicillin G, one merely counts the number of  
23 reaction zones that have changed color to find the  
24 narrow, defined concentration range within which lies  
25 the penicillin G concentration.

Example IV

26 Antibody against a polyvalent antigen (e.g.,  
27 serum albumin) analyte is labeled with peroxidase and  
28 bound to permeable solid medium according to Example  
29 III to form reaction zones in a column. Another batch  
30 of the same or similar antibody is labeled with an  
enzyme such as glucose oxidase. Into the column is  
poured a test sample containing an unknown amount of  
analyte antigen. Through the column is then flowed  
the soluble glucose oxidase-antibody in the presence

1 of glucose plus catalase plus o-dianisidine. The num-  
ber of colored bands resulting is directly related to  
the amount of analyte antigen in the test sample rela-  
tive to the antigen binding capacity of the antibody  
zones. In this example, the antigen first reacts with  
the bound antibody and binds to the antibody, forming  
a predetermined product. The latter, in turn, is de-  
tected by the coupling of the glucose oxidase antibody  
conjugate to available antigenic sites on the antigen  
10 followed by the color forming reaction.

Example V

An analyte or a derivative thereof (e.g., peni-  
cillin-peroxidase) is covalently bound to a permeable  
solid medium according to Example III. An enzyme-la-  
beled receptor (e.g., glucose oxidase-antibody against  
penicillin) is prepared and exposed to the immobilized  
analyte to form the specific binding complex (e.g.,  
immune complex). The assay unit is assembled accord-  
ing to Example III. Subsequent exposure to a test  
20 sample containing an unknown amount of analyte is done  
at elevated temperature (e.g., 60° C) to hasten the  
attainment of equilibrium through competitive binding  
of the immobilized analyte and analyte in the test  
sample with the enzyme-labeled antibody. Analyte in  
the test sample under such conditions will competi-  
tively displace the labeled antibody from the immobi-  
lized analyte. The number of colored reaction zones  
resulting from the procedure is inversely related to  
the amount of analyte in the test sample. These bands  
30 will appear in the terminal or downstream portion of  
the column.

Example VI

Three assay columns with 4 reaction zones each  
were prepared according to Example III, except that  
the top reaction zone was prepared with 75 microliters  
of IgG-agarose suspension (1:1) and the lower 3 zones

1 with 50 microliters. Test samples containing 0, 50 and 200 nanograms Pen-EAC, were placed in different columns, with each test sample containing 200 ng Pen-GO per ml. Flow time for sample application was 20 min. Application of the solution of signal generating reagents produced 2 colored zones with the 0 ng Pen-EAC sample, 3 in the 50 and 4 with the 200 ng sample.

10 A wider and more precise range of analyte content, of course, may be measured by using a larger number of assay zones.

20 In a preferred embodiment, only a single pass through the apparatus of a single liquid material is required. An analyte may be mixed with an analyte derivative, chromogen or other material and flowed through the apparatus to yield an appropriate test result. In a further preferred embodiment, the apparatus is chemically complete in that it includes all reactants and other chemicals necessary or desirable for the quantitative analysis of an analyte; that is, all that is required is that the analyte in a liquid carrier be flowed through the apparatus. Elements of the apparatus that, if combined, would undergo reaction in the absence of the analyte may be maintained in different zones. For example, the bottom-most layer (20.2) of the strip of Figure 2 may contain a reactant physically separated from reactants in the adjacent reaction zone. When the analyte in a carrier liquid is flowed through the layer (20.2), the reactant in this layer together with the analyte and carrier liquid is flowed into the first reaction zone. If desired, a reactant may be provided in the form of a solid and may merely be placed upon the upper layer (18.4) of the column of Figure 1, the reactant being dissolved by and carried with the liquid carrier and analyte into the column.

1 The above-described embodiments are typified by the following Examples VII-IX which also describe and exemplify a preferred format of the invention.

10 This format requires at least two enzymes, one of which is coupled to an analyte to form an analyte derivative and catalyzes a color-forming reaction, and another enzyme that is immobilized in reaction zones which also contain antibody to the analyte, the latter enzyme providing substrate for the color-generating enzyme. In this format, therefore, only a single solution which consists of or contains the analyte test sample is flowed into or through the solid medium after which color develops in the reaction zones, the number of colored zones being directly related to the concentration of analyte in the test sample.

20 Example VII

20 The IgG fraction from rabbit anti-penicilloyl-bovine serum albumin was partially purified by precipitation with 33% saturated ammonium sulfate. This protein was coupled to microcrystalline cellulose by reaction of the cellulose with carbonyldiimidazole in dioxane, followed by washing and then by reaction with the IgG preparation in borate buffer at pH 9.0 at 4 degrees C for two days. The cellulose was then washed extensively with PBS and used for preparation of banded strips. Glucose oxidase was also coupled to microcrystalline cellulose in the same manner. A penicilloyl-peroxidase was prepared by first coupling a polyacrylamide amine to HRP, then reacting penicillin G with that preparation. It is believed that the use of a linear polymer as a spacer for attaching the hapten to the enzyme allows more hapten molecules to be coupled to each enzyme molecule and renders the hapten molecules more accessible for binding to antibody, thus speeding the binding rate. Polyacrylamide was synthesized by dissolving 0.5 gm. of acrylamide in

1 200 ml. of deionized water, degassing, then adding 0.2  
ml. of N,N,N',N'-tetramethylethylenediamine and 0.15  
gm. of ammonium persulfate. This solution was mixed,  
then allowed to sit at room temperature for 30 min.  
then passed through an ultrafiltration membrane,  
dialyzed vs. deionized water and lyophilized. The  
polyacrylamide was then dissolved in 1.0 ml. of 0.2 M  
phosphate buffer at pH 7.7 and 0.3 ml. of 25% glutar-  
aldehyde was added. This solution was incubated at 37  
10 degrees C for 19 hours after which it was passed  
through a Sephadex<sup>\*</sup>G-25 column to remove the excess  
B glutaraldehyde. The void volume fractions which ab-  
sorbed strongly at 230 nm were pooled and added to a  
solution of diaminodipropylamine (0.5 ml. in 2.0 ml.  
of water) at pH 9.0. This solution was allowed to  
react at 4 degrees C over night. The reaction mixture  
was then passed through a Sephadex<sup>\*</sup>G-150 column and  
the fractions that absorbed significantly at 230 nm.  
were divided into four pools of equal volume, the  
20 second of which was coupled to peroxidase (HRP). HRP  
was reacted with 1.25% glutaraldehyde at pH 7.0  
for 15 hours at room temperature. After passing the  
reaction mixture through a Sephadex<sup>\*</sup>G-25 column, the  
HRP-containing fractions were pooled and added to the  
polyacrylamide-diamine preparation, the pH was ad-  
justed to 9.0, and this solution was allowed to react  
at 4 degrees C overnight. The peroxidase-polyacryla-  
mide-diamine was then passed through a Biogel<sup>\*</sup>P-100  
30 column and the void volume fractions were pooled and  
concentrated, then reacted with penicillin. Fifty mg.  
of penicillin G was added to the peroxidase-polyacry-  
lamide-diamine, the pH adjusted to 9.0 and stirred at  
4 degrees C over night. This preparation was then  
dialyzed extensively, then used for the assay.

*\* TRADE MARK*

1 Banded strips were prepared by cutting 0.5 X  
8.0 cm. strips of a polyester film having a hydrophilic  
B surface onto which were glued strips of Whatman\*3MM  
chromatography paper. At one end was glued a 0.5 X 4.0  
cm. long paper strip followed by a 3.5 mm. space. Then  
three one cm. long paper strips were glued onto the  
A <sup>Polyester</sup> <sub>Mylar</sub> strip with 2.0 mm. spaces between them. The  
paper on the <sup>Polyester Film</sup> <sub>Mylar</sub> was wetted with a solution of 0.02%  
o-dianisidine in water. The spaces were then filled in  
10 with a suspension of microcrystalline cellulose pre-  
pared by mixing 50% suspensions of the IgG-cellulose  
and the glucose oxidase-cellulose in a 20:1 ratio.  
The first space was filled with 20 ul. of this suspen-  
sion and the other three spaces each contained 10 ul.  
These strips were air dried, then stored dry until  
used.

20 The strips were developed by placing the end  
with the longer paper spacer into a small vial con-  
taining the developing solution. This solution  
contained peroxidase-polyacrylamide-diamine-penicillin  
(25 ul. of a 0.25 microgram/ml. solution), glucose  
(0.3 ml of a 1.125% glucose solution in 0.2M phosphate  
buffer at pH 6.0) and 10 ul. of dilutions of penicil-  
loyl-aminocaproic acid (EAC) in water. Under these  
conditions, pink bands could readily be observed after  
20-30 min., such that, with no penicilloyl-EAC in the  
developing solution, one band was colored; with 0.4  
micromolar hapten (penicilloyl-EAC), two bands were  
colored; and with 1.0 uM penicilloyl-EAC, all three  
bands were colored.

30 If needed or desired, antibody to peroxidase,  
an HRP-binding lectin or some other binder or inacti-  
vator of peroxidase can be included in the spacer lay-  
ers for the purpose of improving the sharpness or  
decisiveness of zone color determinations. Further-  
more, catalase immobilized in the spacer layers may

\*TRADE MARK

1      permit more rapid color development in the reaction  
      zones without generation of color in the spacer layers.

Example VIII

10     Banded strips are prepared according to Example  
      VII, except that all of the components of the assay  
      except the sample to be tested are incorporated into  
      the strip. The peroxidase-polyacrylamide-diamine-  
      penicillin is dissolved in a solution of between 0.5  
      and 1.0% gelatin containing 2.5% glucose and 0.2 M  
      phosphate buffer at pH 6.0, 0.1 ml. of which is ap-  
      plied to the bottom paper strip and dried. In this  
      example, therefore, the user has only to dip the strip  
      into a solution suspected of containing the analyte,  
      wait for a prescribed time, then read the results by  
      counting the number of colored bands on the strip.

Example IX

20     Assay columns are prepared according to Example  
      III, except that the reaction zones are composed of a  
      mixture of IgG-agarose and glucose oxidase-agarose  
      (20:1). Peroxidase-penicillin (as prepared in Example  
      VII), glucose, o-dianisidine, and phosphate buffer,  
      stored in dry form, are dissolved in 1.0 ml of the  
      test sample which is then added to the column and al-  
      lowed to flow through. The results are read after the  
      prescribed time by counting the number of colored  
      bands on the column. The reagents added to the  
      analyte test sample can be in the form of a small  
      pellet or can be dried onto the under surface of the  
      cap for a small vessel used to measure the volume of  
      sample, etc. In the latter case, the vessel is  
      filled, the cap placed on top, the vessel inverted a  
      few times and the sample is poured into the column.  
      The reagents to be mixed with the sample can even be  
      dried onto a small plug that is stored in the top of  
      the column, in which case they dissolve when the  
      sample is added to the column.

1 Various other enzyme pairs can be used for generating color in the reaction zones. For example, alkaline phosphatase can be immobilized in the reaction zones with beta-galactosidase coupled to the analyte. The use of naphthol-beta-D-galactopyranoside-6-phosphate as substrate for the alkaline phosphatase results in the generation of naphthol-beta-D-galactopyranoside, which is hydrolyzed by beta-galactosidase to produce naphthol which in the presence of a diazonium salt results in a colored product in the reaction zones.

10

20 The accuracy and reliability of the apparatus of the invention depends to some extent upon how readily or easily the generation of color or other detectable change in the different reaction zones may be ascertained. A reaction zone in the direction of analyte flow desirably should show detectable changes only when a significant, minimum quantity of analyte or other material being detected has passed through the preceding reaction zone; since the physical nature of the apparatus often does not permit reaction to go fully to completion in each such zone, a small "tail" e.g., trace, amount of material may flow into successive zones and may be marginally detected in such zones to yield readings that are difficult to interpret. One may largely avoid this problem, however by several means. Detectors may be employed that are sensitive only to minimum concentrations of a chemical moiety to be detected. For example, one may utilize o-phenylene diamine in place of o-dianisidine as a chromophore in the above examples, the former being less sensitive. Another method involves the placement in spacer layers or, less desirably, in reaction zones, of small quantities of "scavenger" reactants capable of immobilizing or deactivating

30

1 trace amounts of materials, as exemplified in Example  
VII. This enables the sensitivity and operation of  
the apparatus to be tailored as desired to particular  
analyses. Control of sensitivity and reliability also  
may depend upon the concentration of the reactant in  
the solid reaction zones, and the solubility of mate-  
rials such as the colored product in some analyses.

10 While a preferred embodiment of the present in-  
vention has been described, it should be understood  
that various changes, adaptations and modifications  
may be made therein without departing from the spirit  
of the invention and the scope of the appended claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR  
PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Apparatus for the quantitative analysis of an analyte in a fluid, comprising a fluid-permeable solid medium defining a path for fluid flow and having a predetermined number of successive, spaced reaction zones in the path of flow, the reaction zones having immobilized therein predetermined quantities of a reactant capable of competitively reacting with or competitively binding to the analyte or an analyte derivative to result in the formation of a predetermined product; the analyte or its derivative, the reactant or the predetermined product in the reaction zones being detectable and the number of reaction zones in which such detection occurs indicating quantitatively the amount of analyte in said fluid.
2. The apparatus of claim 1 wherein said fluid-permeable solid medium includes spacer sections in flow communication with but separating said reaction zones.
3. The apparatus of claim 1 wherein the fluid-permeable medium in the reaction zones has immobilized therein an antibody specific to an analyte to be quantitatively analyzed.
4. The apparatus of claim 1 including detection means for detecting, in the reaction zones, the analyte or its derivative, the reagent, or the predetermined product.
5. The apparatus of claim 2 wherein said reaction zones are vertically spaced in a column.
6. The apparatus of claim 2 wherein the reaction zones are spaced along the length of a strip of solid, fibrous material through which fluid is drawn by capillary action.
7. The apparatus of claim 2 wherein the reaction zones are in the shape of concentric rings and the spacer sections are in the shape of concentric rings sandwiching between them the reaction zones.
8. The apparatus of claim 2 including flow rate controlling means for controlling the rate of fluid flow along the fluid flow path.
9. Method for the quantitative analysis of an analyte in a carrier liquid, comprising the steps of:  
providing a liquid-permeable solid medium defining a flow path and having a predetermined number of successive, spaced reaction zones in

the path of flow, said reaction zones having immobilized therein a reactant competitively reactive with or competitively binding to the analyte or an analyte derivative or both to result in the formation of a predetermined product;

flowing said liquid along the flow path and sequentially through the spaced reaction zones; and

detecting the presence of analyte, analyte derivative, reactant or predetermined product in the reaction zones;

the amount of analyte in the determining liquid being a function of a number of zones in which such detection occurs.

10. The method of claim 9 wherein said analyte and said reactant are ones of a specific ligand-antiligand binding pair.

11. The method of claim 10 wherein said reactant includes an antibody specific to said reactant.

12. The method of claim 9 wherein reaction between said analyte and reactant renders the analyte, analyte derivative, reactant or predetermined reaction product nondetectable during said detection step.

13. The method of claim 9 wherein said liquid contains said analyte and a known concentration of said analyte labeled with a chemical moiety, the presence of which chemical moiety is detected in said detection step.

14. The method of claim 13 wherein the chemical moiety is an enzyme.

15. The method of claim 13 wherein the liquid is milk, the analyte is penicillin and the reactant includes an anti-penicillin antibody.

16. The method of claim 15 wherein the liquid includes a known concentration of an enzyme-labeled penicillin.

17. Method for the quantitative determination of an analyte in a fluid containing a known quantity of a labeled analyte, comprising the steps of:

providing a fluid-permeable solid medium defining a fluid flow path and having immobilized therein a reactant reactive with the labeled analyte in competition with the analyte to result in the formation of a predetermined product;

flowing said fluid along the flow path; and

detecting the presence of the labeled analyte or the predetermined product along the flow path;

the length of the flow path in which such detection occurs being a

function of the amount of analyte in the fluid.

18. Method for the quantitative determination of penicillin in a liquid containing a known quantity of a labeled penicillin, comprising the steps of: providing a liquid-permeable solid medium defining a liquid flow path and having immobilized therein a reactant including an antibody to said penicillin and to said labeled penicillin and reactive competitively therewith to result in the formation of a predetermined product; flowing said liquid along said flow path; and detecting the presence of the labeled penicillin or the predetermined product along the flow path; the length of the flow path in which such detection occurs being a function of the amount of penicillin in the liquid.

19. The apparatus of claim 1 including means in the reaction zones or spacer sections for suppressing therein the detection of trace amounts of the analyte or its derivative, the reactant or the predetermined product.

20. The apparatus of claim 3 wherein said reaction zones are particularly adapted to change colour upon contact therein with an analyte derivative that is a conjugate of an analyte and an enzyme, the reaction zones having immobilized therein another enzyme, one of said enzymes being capable of generating substrate for the other said enzyme.

21. The apparatus of claim 20 wherein said analyte-enzyme conjugate is contained in a layer upstream from a reaction zone, whereby flow of an analyte in a fluid carrier through said layer carries the analyte conjugate into said reaction zone.

22. The method of claim 9 wherein only a single liquid containing an analyte is flowed in said flow path.

23. The method of claim 22 wherein said liquid consists essentially of the analyte and carrier liquid.

24. The method of claim 22 wherein said liquid includes an analyte and an analyte derivative.

25. The method of claim 9 including the step of suppressing the detection of trace amounts of analyte, analyte derivative, reactant or predetermined product.

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